Flow Cytometry Shared Resource

Mission

• To provide professional, efficient, timely and reasonably priced service.
• To provide continuous education and consultation in the utilization of the facility.
• To provide support for CTRC programs

Services

• Multicolor analyses and sorting, phenotyping, cloning of subpopulations of lymphocytes, tumor cells and (cancer) stem cells
• Detection of apoptosis-related changes
• Measurements of nitric oxide, hydrogen peroxide, free radicals, pH; Ca++ fluxes and fluxes of different dyes
• Quantitation of multiple cytokines using fluorescent microspheres for multiplex detection of cytokines and chemokines
• Assistance in writing flow cytometry methodologies for publications and grant applications
• Interactive web site for scheduling time, looking up methodologies and general information about the facility
• Figure-making services of publication quality
• Training sessions for self operation

Key Equipment

1.) 3-laser (405nm, 488nm, 633nm), 9-color FACSaria cell sorter capable of up to 20,000 events/sec. Upgrade to 5-laser (375nm, 405nm, 488nm, 1000nm), 9-color Cell sorter capable of up to 20,000 events/sec. Upgrade to 5-laser (375nm, 405nm, 488nm, 1000nm), 9-color
2.) 3-laser (405nm, 488nm, 633nm), 8-color LSR-II flow cytometer. 3.) 2-laser (488nm, 633nm) 4-color FACSCalibur Analyzer (2)

Scientific Director: Vivienne I. Rebel, M.D., Ph.D.
Technical Director: Benjamin J. Daniel, Ph.D.
Flow operator: Karla Gorenna

Personnel

Common Applications From Users at UTHSCSA

Flow Cytometry Is The Standard Method To Study Regulatory T Cell (Treg) Phenotype And Function.

To study Tregs, functional assays must be performed. A typical functional assay is described as follows: (1) Suspected Treg population is cell sorted into a highly purified population. (2) Non-Treg cell are stained with a dye that measures cell viability (7-AAD), and activated (7-AAD positive cells) and stimulated (7-AAD negative cells) by addition of mitogens or cytokines. (3) Sorted cells are then added to the stimulated, non-Treg population to see if the Treg suppress cell proliferation. (4) Graphical Overview of stimulated cells from panel B (red line) and panel C (green line) shows that the sorted cells Treg suppress proliferation.

Data was acquired and sorted in the UTHSCSA Flow Cytometry Core and generously donated by Sue Stacy and Ellen Knoss.

Multi-Parameter Sorting Yields Highly Proliferated Stem Cell and Progenitor Cell Populations Which Can Then Be Used in Future Experiments

Bulky glands were purified by enzymatically digested and isolated into single cell suspensions. Cells were then stained with antibodies to CD8 (Ly1) and CD4 (Ly18) and divided into 3 subsets: (A) functional T cells; (B) immune reactive cells; and (C) sorted cells. (C) Sorted cells were then added to the stimulated, non-Treg population to see if the Treg suppress cell proliferation. (B) Graphical Overview of stimulated cells from panel B (red line) and panel C (green line) shows that the sorted cells Treg suppress proliferation.

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Flow Cytometry can be used to study several phosphorylated proteins in a given cell population simultaneously.

Peripheral blood mononuclear cells were isolated by homopaque density-gradient centrifugation. Monocytes were then purified by magnetic bead selection. Monocytes were stimulated with LPS at 1 µg/mL for varying length of time and stained for multi-color flow cytometry. The levels of p38 phosphorylation in the monocytes were determined by FACS analysis (A) and Western blotting (B). The levels of ERK phosphorylation in the monocytes were determined by FACS analysis (C) and Western blotting (D).

Data was acquired and sorted in the UTHSCSA Flow Cytometry Core and generously donated by Hany Liu and Nicholas Mai.


MiR-155 limits the cytotoxic effects of TGF-b1 and BMP2/4 in DLBL. DLBL cell lines constitutively expressing miR-155 were significantly more resistant (P < 0.05) to TGF-b1 (A) and BMP2/4 (B) than their isogenic counterparts. TGF-b1 doses were 1 ng/mL (ly1) and Ly18 and 2 ng/mL (ly19) see Fig. S4A for the complete dose range. Data shown are mean ± SEM of the percentage of cells exposed to TGF-b1 or BMP2/4, normalized by vehicle-treated cells. (C) Cell cycle analyses show G0/G1 arrest after TGF-b1 exposure in DLBL. (D) Real-time RT-PCR quantification of p21 induction by TGF-b1 (2.5 ng/mL). MiR-155 expression significantly blocked TGF-b1-mediated induction of p21 in DLBL (P < 0.05). Data shown are mean ± SEM of the percentage of cells exposed to TGF-b1 normalized by vehicle-treated cells. TGF- b1 consistently did not induce p21 expression in the Ly19 cell line.

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Contact Information

Location Flow Cytometry Core: 5.044V (Microbiology Dept.)
Telephone: (210) 567-3911
Website: http://www.micro.uthscsa.edu/facs/index.asp